REMARKS/ARGUMENTS

Reconsideration of this application is respectfully requested.

As required, a new more descriptive title has been effected by the above amendment.

In response to the Examiner's comments about the Abstract, it is noted that this case was filed with a copy of the cover sheet of the published International application which included an Abstract. That Abstract has been amended above so as to put it into more traditional US format.

In response to the rejection of claims 1-9, 13, 24 and 27 under 35 U.S.C. §112, second paragraph, these claims have also been reviewed and amended where it is appears appropriate to obviate the Examiner's stated grounds of objection/rejection.

However, the Examiner is asked to reconsider a few of these objections. For example, the phrase "characteristic cycle time" is explicitly defined in the paragraph bridging pages 2 and 3 of the specification. It is also believed to be understood in the same fashion by those skilled in the art. Similarly, "active elements" is also clearly described throughout the specification, for example, at pages 22, 38 and 39 so as to include fluorescent materials, phosphorescent materials and luminescent materials. As those skilled in the art will appreciate from the applicant's specification, the described

and claimed invention may be utilized in connection with the emission and detection of any suitable quanta (e.g., see page 40, final paragraph). The active species may comprise a simple fluorophore or a combination of elements. In other words, while the phrase "active elements" is a broad term, it is not "vague and indefinite" or "unclear" in its intended meaning.

Similarly, the use of the word "some" at line 3 of claim 1 is not believed to be in any way vague or indefinite. The word "some" is a commonly used word in the English language meaning one or more.

A more particular description of "modifying moiety" as used in some of the claims is also provided in the specification (e.g., see page 28, last full paragraph) -- and is also believed to be of definite understanding by those skilled in the art when encountered in the context of applicant's specification and claims.

Accordingly, all outstanding formal issues are now believed to have been resolved in the applicant's favor.

The rejection of claims 1-4, 26 and 29 under 35 U.S.C. §102 as allegedly anticipated by Soper is respectfully traversed -- as is the additional rejection of claims 3-28 under 35 U.S.C. §103 as allegedly being made "obvious" based on Soper in view of "each of" Lakowicz '169 and Walt '477.

The Examiner bases all rejections upon the premise that the term "characteristic cycle time" is synonymous with the term "fluorescent life time". This premise is incorrect. Fluorescence lifetime is a fundamentally different physical parameter than the characteristic cycle time. Soper (and Lakowicz, '169) simply relate to measurement of the fluorescent lifetime, not the fluorescent characteristic cycle time.

The Examiner appears to have confused the concept of fluorescent lifetime with fluorescent characteristic cycle time. Below is an explanation of the differences.

Fluorescence is the emission of radiation from a material during illumination by radiation of higher frequency. In other words, the material absorbs light at a first wavelength (i.e., energy), and then subsequently emits a photon at a second, longer wavelength (i.e., lower frequency, and hence energy). The fluorescent lifetime is the average time the material remains in an excited energy state, before the material emits the lower energy photon. For example, the Examiner's attention is directed towards the "Background of the Invention" section of Walt ('477), which also explains this phenomenon.

Figure 4 of the present application (along with the associated description on pages 15 and 16) describe the energy states of a simple fluorescent material. The fluorescent material is excited from the ground state 7 to the upper energy state 8 by the absorption of energy from an outside source, i.e., by the absorption of a photon with energy

corresponding to the difference in energy level between the state 7, 8. The fluorescent material then decays to a lower energy state (9) by emission of a (lower energy) photon. The material will then decay from the lower energy state 9 to the ground state 7.

The fluorescent lifetime is the average period of time the material remains in the excited state before it emits the photon, i.e., the average amount of time the material spends in energy state 8, prior to transition to energy state 9.

As noted by the Examiner, page 2, lines 29 - 30 of applicant's specification defines the characteristic cycle time as the time taken for an active element to return to a ground state following excitation to an excited state. In the relatively simple system illustrated in Figure 4, the cycle time will correspond to the average time the material spends in energy states 8 and 9. In other words, in this particular example, the characteristic cycle time is equal to the fluorescence lifetime plus the average time the material spends in lower energy state 9.

The energy states illustrated in Figure 4 are obviously provided by way of example only. Different system can have different arrangements of energy states. However, as fluorescence relates to the emission of a photon of lower energy than that utilized to excite the material, there will always be additional energy states, besides the excited energy state. Thus, the characteristic cycle time is not, and will never be, the same as the fluorescence lifetime.

Soper does not anticipate the present claims, as Soper discloses a method and apparatus for measuring fluorescence lifetime (e.g., see abstract). Soper achieves this by using a conventional technique.

A laser pulse is utilized to excite the relevant material, and then the time difference between the laser pulse and the emission of the photon from the material is recorded (e.g., see page 1763, column 2, paragraph 3). Thus, Soper discloses using time resolved fluorescence (the time of the emitted photon arrival after the excitation laser pulse) to detect fluorescent lifetime. It should also be noted that this is one of the known techniques for measuring the fluorescent lifetime suggested in Lacowicz (e.g., see claim 5). It is further noted that Lacowicz discloses using an alternative technique ("phase-modulation fluormetry", e.g., see claim 4), which is one of he techniques acknowledged in paragraphs 4 and 5 on page 1 of the present application (and described in detail in the book by J.R. Lacowicz, acknowledged in those paragraphs).

Thus, the prior art documents that the Examiner considers most relevant describe only fluorescence lifetime measurements, and not fluorescence cycle time measurements. Further, such measurements utilize time varying (i.e., phase-modulated or pulsed) light sources, to excite the relevant material to allow the measurement to occur.

The present invention is directed to a method and apparatus for determining a characteristic cycle time of the sample. None of the prior art documents disclose, or even suggest, the measurement of such a characteristic cycle time.

The claimed method includes the step of exciting active elements (e.g., fluorophores in the case of fluorescence) in the sample with sufficient intensity that at least some of the active elements are re-excited to an excited state substantially immediately following relaxation to the ground state. None of the cited documents relating to fluorescence lifetime disclose such a concept, which is an essential element to the present invention.

The optimum way of providing such an excitation for a fluorescent system is to provide a continuous beam of light, i.e., from a continuous wave (CW) laser, preferably of uniform intensity, so as to provide a constant rate of photon collision (e.g., see page 13, paragraph 2 of the present specification).

As described in page 14, paragraph 1 to page 14, paragraph 3, pulse light <u>may</u> be used to measure the fluorescence cycle time. Such pulses should have a duration much <u>longer than the characteristic cycle time</u> of the fluorophores comprising that sample. This contrasts with Soper, which uses a technique in which it is desirable that the pulses are a lot <u>shorter than the fluorescence lifetime</u> (e.g., see right column of page 1762, which indicates the minimization of the cavity length to adjust the laser pulse width to less than

50 picoseconds, and compare with left column of pages 1768, paragraph 2, which indicates the apparent lifetimes of around four nanoseconds, i.e., the pulse width is at least several orders of magnitude less than the life time measurement. This is necessary, so as to prevent the emitted photons from the fluorophore being swamped at the detector by photons from the pulse, in the measurement technique disclosed in Soper.

Finally, and significantly, it can be observed that in Soper a shorter wavelength (higher energy) radiation beam is utilized to excite each species of dye molecule, with fluorescence subsequently occurring at a higher (lower energy) wavelength. For example, it will be observed that two excitation wavelengths (one at 532 nm, the other at 585 nm) are focused through the sample (flow cell). The 532 nm wavelength is utilized to excite the fluorescent dye R6G (which fluoresces at a peak wavelength of 550 nm), while the 585 nm excitation wavelength is utilized to excite the other dye TR, which fluoresces at a peak wavelength of 605 nm (e.g., see Figure 2 of Soper). As mentioned within the abstract, the technique was utilized to efficiently distinguish between the two species of dye molecules, on the basis of differences in their emission spectra. The fluorescent lifetime measurement was only made in respect of TR (right hand column of page 1767, paragraph 2). As indicated in Figure 2, the fluorescent emission peak of TR is around 605 nm (i.e., at a wavelength of lower energy than both excitation wavelengths). As the fluorescence lifetime was measured, it will be appreciated that the characteristic cycle time of the dye was not measured.

In other words, neither Soper nor any of the cited prior art documents disclose the concept of measuring cycle time, let alone the concept of measuring cycle time as claimed within the present independent claims.

The Examiner also notes that Soper discloses correlation of a detected signal. Soper does indeed appear to disclose the concept of correlation of a detected signal in the cited portion (page 1765, right column, lines 7-11). However, as indicated on page 1764, left column, paragraph 2, auto-correlation is utilized to investigate cross-talk. The autocorrelation signal is <u>not</u> utilized to determine the lifetime measurements. As disclosed in the right hand column of page 1763, paragraph 3, lifetime measurements are determined by recording the time difference between the laser (excitation) pulse and the arrival of the (fluorescence) photon. Thus, Soper does not disclose the concept of correlating the detected signal with itself to derive the characteristic cycle time (i.e., the subject matter of claim 2).

Further, as the characteristic cycle time is not determined by Soper (only the fluorescence lifetime), then the subject matter of claims 2-4 is novel for many reasons.

The Examiner also rejects claim 26 as lacking novelty based upon Soper. However, the Examiner does not explain his reasoning in any detail. In fact, claim 26 includes the concept of measuring the lifetime of the excited state (e.g., he fluorescent lifetime), and then subtracting that lifetime from the characteristic cycle time. Based

upon the simple system illustrated in Figure 4 of the present application, this would correspond to first determining the cycle time, then subtracting the lifetime of the excited state 8 from the cycle time, i.e., the remainder would be the lifetime of energy state 9. As the Examiner appears to (incorrectly) consider the characteristic fluorescence cycle time as being the fluorescence lifetime, it is unclear as to why the Examiner considers Soper to disclose the subject matter of claim 26. Based upon the Examiner's reasoning, one would appear to be subtracting the fluorescence lifetime from the fluorescence lifetime!

Accordingly, this entire application is now believed to be in allowable condition and a formal Notice to that effect is respectfully solicited.

Respectfully submitted,

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